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Treatment of *Klebsiella pneumoniae* Respiratory Tract Infection of Squirrel Monkeys with Aerosol Administration of Kanamycin

Richard F. Berendt PhD; Robert D. Magruder Frank R. Frola, BA

SUMMARY

The therapeutic efficacy of IM-administered kanamycin was compared with the efficacy of aerosol-administered kanamycin in *Klebsiella pneumoniae*-infected squirrel monkeys. Differences in mortality or morbidity were not seen with equivalent dosages of antibiotic ranging from 15 to 6.9 mg/kg of body weight/day. Seemingly, the IM route of kanamycin administration was as effective as the aerosol route for therapy.

Aerosol administration of kanamycin was more effective in preventing respiratory *Klebsiella pneumoniae* infection in mice and squirrel monkeys, if given prior to exposure, than were similar doses of the antibiotic given IM.[1] Administration of this antibiotic given after infection was more effective than was IM administration for the therapy of respiratory *K pneumoniae* infection in mice.[2] Because the histopathologic features of *K pneumoniae* infections in mice differ from those observed in squirrel monkeys,[3] the present study was undertaken to compare the therapeutic effect of aerosol administration of kanamycin with IM administration in a nonhuman primate.

Materials and Methods

Test Organism—Techniques for growing, storing, and enhancing the virulence of the A-1D strain of type 1 *K pneumoniae* have been described.[2] Inocula for intratracheal infections of squirrel monkeys, containing 700 organisms (1 LD_{50}) or 7,000 organisms (10 LD_{50}) were prepared as previously described.[4]

Test Animals—Healthy, juvenile, male squirrel monkeys (*Saimiri sciureus*, weighing 0.5 to 1.0 kg) were used. They were housed individually in wire-bar cages and were allowed free access to commercial monkey feed and water. Their diet was supplemented several times weekly with fresh fruit. During experiments, the fruit was withheld and food intake was monitored.

Treatment Techniques—All kanamycin solutions were prepared from 78% kanamycin *base*[a] *dissolved in 0.085 M sodium* citrate solution adjusted to pH 4.5 with concentrated sulfuric acid. Aerosol treatment with kanamycin was accomplished in an exposure box.[1] Sample collection of aerosol preparations and determination of the dosage of antibiotic that was inhaled were carried out as previously described.[4]

Clinical and Laboratory Tests—Rectal temperature, hematocrit, total leukocyte count, respiratory rate, food intake, and weight were recorded daily. Also, on a daily basis, the monkeys were observed for dyspnea, coughing, and any changes in activity. Overall mortalities were calculated at the end of the experimental period.

Experimental Designs—Preliminary Experiments—The dosage of kanamycin used for aerosol and IM treatment was 11.25 mg/kg/day. Because information on recommended dosage for monkeys could not be found, the amount recommended by the manufacturer for dogs and cats was used. One half was administered in the morning, the balance was given about 6 hours later. The number of *K pneumoniae* organisms instilled was 1 LD_{50} (700 cells). This number was chosen on the premise that clinical response and mortality could be studied simultaneously. Because of constraints imposed by the numbers of monkeys available at any one time, this experiment was conducted in several phases. In the 1st phase, 5 infected monkeys were treated by IM injection twice daily for 5 days, beginning 24 hours after infection. Five infected monkeys were not given therapy (*K pneumoniae*-controls), 5 were given IM therapy but were not infected (kanamycin controls), and 5 were observed with no treatment at all (normal controls). At least 3 base-line observations were completed on each monkey; observations were continued for 7 days after infection. This experiment was repeated 3 times. Normal controls for each replication were used as infected controls in the next experiment to reduce the number of monkeys required.

The aerosol-therapy trials were performed in a similar manner, but the number of monkeys in each group was limited to 4 (the number that could be accommodated in the treatment device) and only 2 replications were carried out.

Dose Response Experiments—To determine the effect of the dose of kanamycin administered and to calculate the median effective dose (ED_{50}), 4 groups of monkeys were treated IM with selected doses and 4 groups of 3 monkeys were similarly treated by aerosol administration. Treatment was initiated 24 hours after infection and was continued for 5 days. The infecting dose was 7,000 organisms (10 LD_{50}). Four additional monkeys were infected with *K pneumoniae* but were not treated, and 4 were used as normal controls. Kanamycin controls (IM and aerosol) were not included because no change was not noticed in the preliminary experiment.

Results

Preliminary Experiments—The mortality data from the 1st experiment are given in Table 1. Deaths occurred in the *K pneumoniae*-control groups in which the mortality was

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From the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701.

[a] Bristol Laboratories, Syracuse, NY.

48%; deaths occurred over the 48-hour period between 48 and 96 hours. Four of the laboratory and clinical findings are presented (Fig 1). These observations indicated that the 2 treatment techniques were approximately equally effective. Interpretation of the clinical responses of the infected control monkeys is subject to uncertainties because the number of monkeys declined from 23 at 24 hours to 12 at 96 hours. The reason for persistence of increased respiratory rates in the aerosol-treated animals in comparison with rates in infected controls was not immediately apparent, but may have resulted from an interaction of drug and *K pneumoniae* in the lungs. The noninfected aerosol-treated animals did not show this reaction.

Klebsiella pneumoniae organisms were cleared from blood more rapidly after IM than after aerosol treatment. Conversely, aerosol preparation caused a more rapid clearance of organisms from the oropharynx than did IM treatment (not shown).

Dose Response—The dose response data are shown in Table 2. Unfortunately, these data are somewhat equivocal. Only 1 of 3 monkeys treated IM died at the lowest dose (6.9 mg/kg). The 3.8 mg/kg dose was ineffective in aerosol-treated monkeys, but ED_{50} values cannot be calculated with 0% or 100% effects. Therefore, it can only be concluded that the aerosol ED_{50} lies between 6.9 and 3.8 mg/kg and the IM ED_{50} is < 6.9 mg/kg.

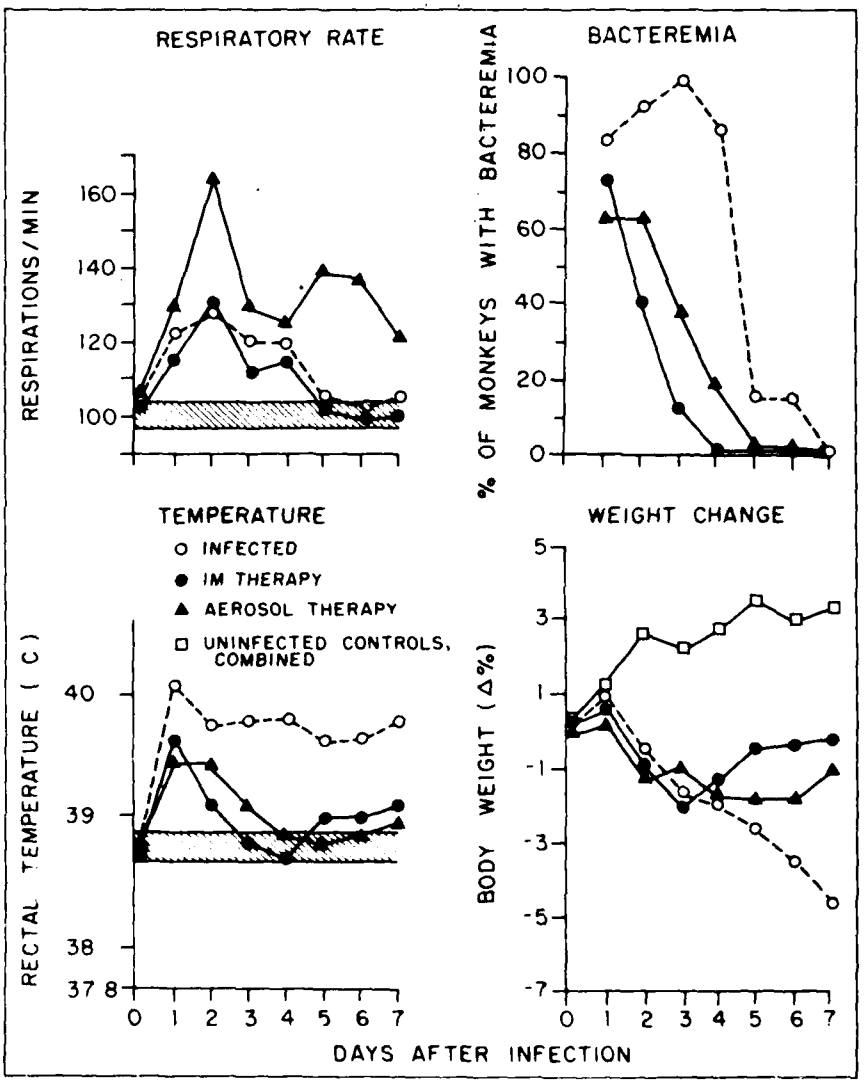

Fig 1—Effect of aerosol and IM kanamycin therapy on selected laboratory and clinical measurements in *Klebsiella pneumoniae*-infected squirrel monkeys. The cross-hatched bars represent the mean ± SEM of combined noninfected control monkeys.

TABLE 1—Mortality of Squirrel Monkeys Infected with an LD_{50} Dose of *Klebsiella pneumoniae* and Treated with Kanamycin*

Group	n	Dead	(%)
Noninfected kanamycin controls (aerosol)	8	0	0
Noninfected kanamycin controls (IM)	15	0	0
Normal controls	23	0	0
K pneumoniae controls	23	11	48
Infected-treated IM	15	0	0
Infected-treated by aerosol	8	0	0

* Treatment initiated 24 hours after infection and continued for 5 days. Dosage of kanamycin was 11.25 mg/kg of body weight/day.
n = No. of monkeys used.

TABLE 2—Mortality of *K pneumoniae*-Infected Squirrel Monkeys Treated with Graded Doses of Kanamycin*

Kanamycin (mg/kg/day†)	Dead/total No. infected	Mean time to death (days)
None (Noninfected controls)	0/4	NA
None (*K pneumoniae*-infected controls)	4/4	2.0
IM		
6.9	1/3	7.0
12.5	1/3	7.5
15.0	0/3	NA
22.5	0/3	NA
Aerosol		
3.8	3/3	3.3
6.9	1/3	6.0
9.0	1/3	7.0
15.0	0/3	NA

* Challenge-exposure inoculation was 7,000 organisms (10 LD_{50}). †One half of the selected dose was administered at each of 2 periods daily for 5 days.
NA = not applicable.

Discussion

In contrast to the previously reported therapeutic superiority of the aerosol route over the IM route in mice[4] and even though prophylactic aerosol administration is clearly more effective than is IM administration in monkeys, the results of the present study do not support the hypothesis that aerosol therapy is more effective than IM treatment of *K pneumoniae* infection in monkeys. Any difference in survival was not noticed, and the severity of illness was about the same, regardless of the route of treatment.

Despite the fact that clearance of kanamycin from the lungs is more rapid after IM than after aerosol administration,[1] sufficient antibiotic was present to promote survival after IM injection. Based upon the present data, the number of monkeys used was too small to permit ED_{50} calculations, although it can be said that 15 mg/kg/day for 5 days should be an effective dose by either route. It may

be theorized that other aminoglycoside antibiotics will act similarly because the degree of binding in the lungs is apparently similar.[5]

Aerosol therapy seems to be equally effective in treating infections in monkeys and mice, but IM administration of therapy is more effective in monkeys than in mice. Probably the increased efficacy of IM injection of kanamycin is due to the difference in pathogenesis of *K pneumoniae* in monkeys and mice.[2] This organism causes bronchopneumonia in mice and lobar pneumonia in monkeys.[3] For reasons that are presently not understood, IM injection is as effective as are aerosol preparations in treating lobar pneumonia; the clinician must choose the route of administration of therapy based upon this rationale.

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